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REMARKS

Claims 1-20 are pending in the instant application. The rejections set forth in the Office Action are traversed by argument below.

1. Rejection of claims 1 and 2 under 35 U.S.C. § 102

The Office Action asserts a rejection of claims 1-20 under 35 U.S.C. § 102(e), as being anticipated by U.S. Patent No. 6,433,145 (the '145 Patent). The Action accurately states that the '145 Patent discloses an amino acid sequence (i.e., the amino acid sequence set forth in SEQ ID NO: 2 of the '145 Patent) that is identical to the amino acid sequence set forth of SEQ ID NO: 5 of the instant application. The Action also states that the '145 Patent discloses variants; pharmaceutically acceptable carriers; the fragment set forth in SEQ ID NO: 6 of the instant application; derivatives, including polymers; fusion proteins; and expression in eukaryotic and prokaryotic cells.

Applicants submit a Declaration under 37 C.F.R. § 1.131 establishing conception of the subject matter of the claims rejected under 35 U.S.C. § 102(e) prior to the effective date of the reference on which the rejection is based, as well as establishing that the subject matter of the rejected claims was diligently reduced to practice. Applicants note that due to the unavailability of some of the named inventors, Applicants' representative was unable to secure an executed Declaration. However, Applicants' representative will secure and promptly submit an executed Declaration containing the signatures of all three named inventors. Applicants contend that because the Declaration sufficiently establishes the conception of the subject matter of the claims prior to the effective date of the '145 Patent, and further, establishes that the subject matter of the rejected claims was diligently reduced to practice, the Declaration is sufficient to overcome the rejection of claims 1-20 under 35 U.S.C. § 102(e) as being anticipated by the '145 Patent. Applicants, therefore, respectfully request that this ground of rejection be withdrawn.

CONCLUSIONS

If Examiner Andres believes it to be helpful, she is invited to contact the undersigned representative by telephone at 312-913-0001.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff

Dated: June 10, 2004

Reg. No. 48,710



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE (Case No. 99-372-F)

| In re Application of: Welcher et al. | PATENT) |
|--------------------------------------|----------------------------------|
| Serial No.: 09/927,850 |) Before the Examiner: J. Andres |
| Filed: August 10, 2001 |) Group Art Unit: 1646 |
| For: Interferon-Like Molecules |) |
| and Uses Thereof |) |
| Commissioner for Patents | |
| P O Roy 1450 | |

Sir:

Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R § 1.131

We, Andrew A. Welcher, residing at 1175 Church Street, Ventura, California; Duanzhi Wen, residing at 3885 Campus Drive, Thousand Oaks, California; and Michael Kelly, residing at 790 San Doval Place, Thousand Oaks, California; hereby declare:

- 1. We are named co-inventors on United States Application No. 09/927,850, filed on August 10, 2001.
- 2. The invention disclosed and claimed in the instant patent application was conceived in the United States by us before July 21, 1998 and was then diligently reduced to practice.
- 3. Accompanying this Declaration are photocopies of forty-one (41) pages from our laboratory notebook showing conception of our invention before July 21, 1998. Specifically, the photocopies of our laboratory notebook show that a genomic cloning approach was used to identify the nucleic acid sequence of human interferon-like polypeptide (see page 34 of laboratory notebook). Three genomic clones were identified as containing nucleic acid sequences encoding at least a portion of human interferon-like polypeptide (i.e., clones 2, 6, and 7; see page 40). The nucleic acid sequences from these clones were isolated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was determined to contain a partial nucleic acid sequence for human interferon-like polypeptide and another genomic clone (i.e., clone 6) was determined to contain a full-length nucleic acid sequence for human interferon-like polypeptide (see page 62). The amino

acid sequence of human interferon-like polypeptide was determined from the latter nucleic acid sequence.

- 4. The dates on the laboratory notebook pages have been redacted from the photocopies. However, the dates are before July 21, 1998, the date on which U.S. Provisional Application No. 60/093,643 was filed, from which U.S. Application No. 09/487,792 claims the benefit of priority, from which U.S. Patent No. 6,433,145 issued on August 13, 2002.
- Also accompanying this Declaration are photocopies of ten (10) pages from a Research Summary showing that the invention disclosed and claimed in the instant patent application was diligently reduced to practice. Specifically, the photocopies of the Research Summary show that experiments were performed in order to determine the function of protein encoded by the nucleic acid sequence described in paragraph 3 above, and that once the function of the protein had been determined, a Research Summary was prepared and submitted to the legal department of Amgen Inc., the assignee of the instant application. More particularly, photocopies of the Research Summary show that several versions of the human and rat IFN-L proteins were produced in a mammalian expression system (see page 7) and that rat IFN-L:Fc fusion protein treatment of several cell lines was found to cause phosphorylation of cellular proteins (see page 10).
 - 6. The dates on the Research Summary pages have been redacted from the photocopies.
- 7. We hereby declare further that all statements made herein by each of us to our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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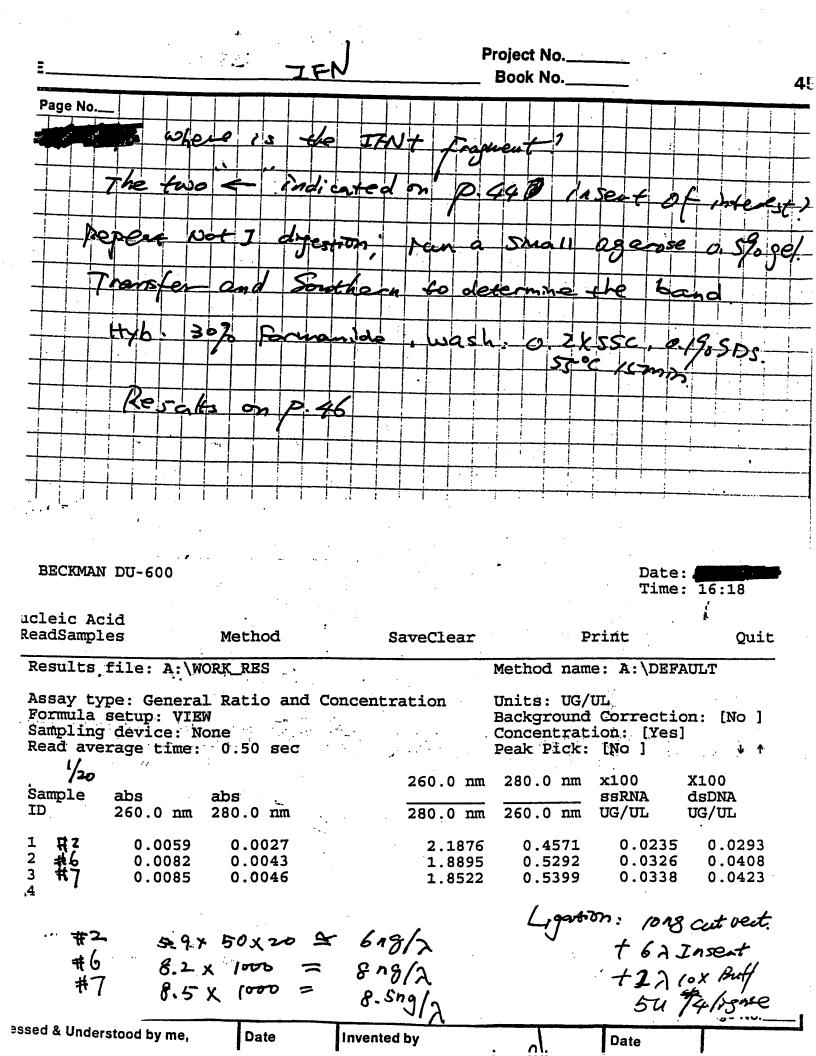
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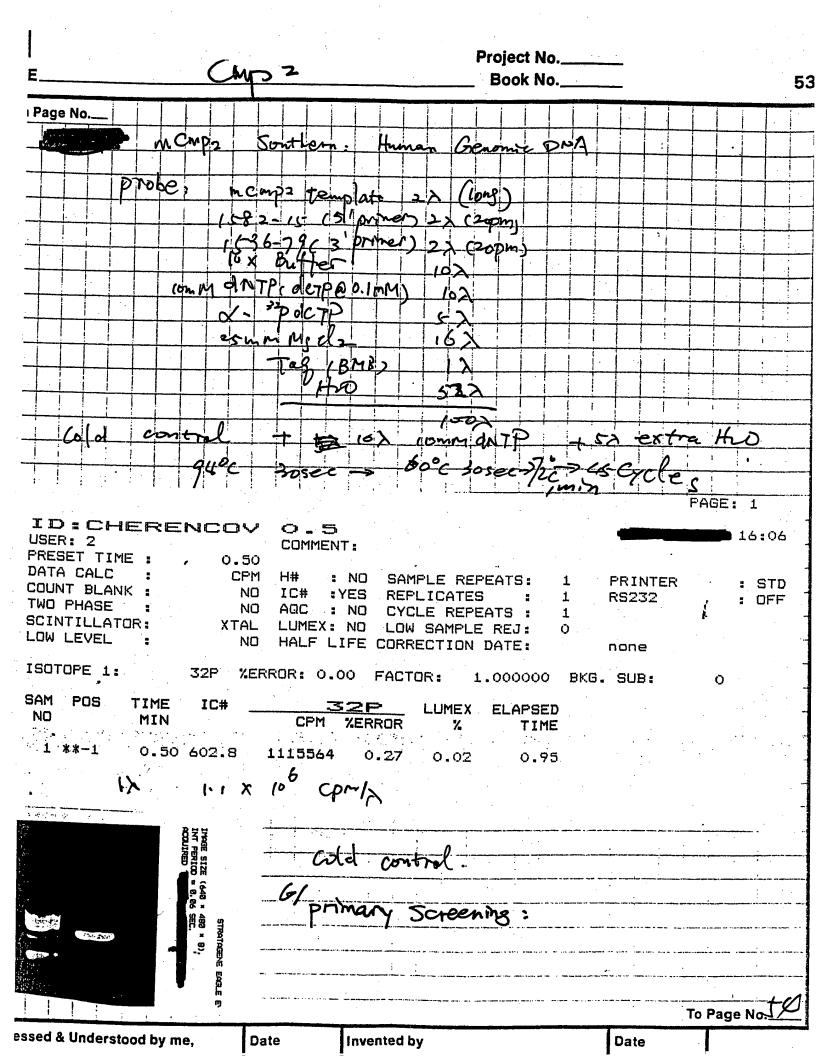
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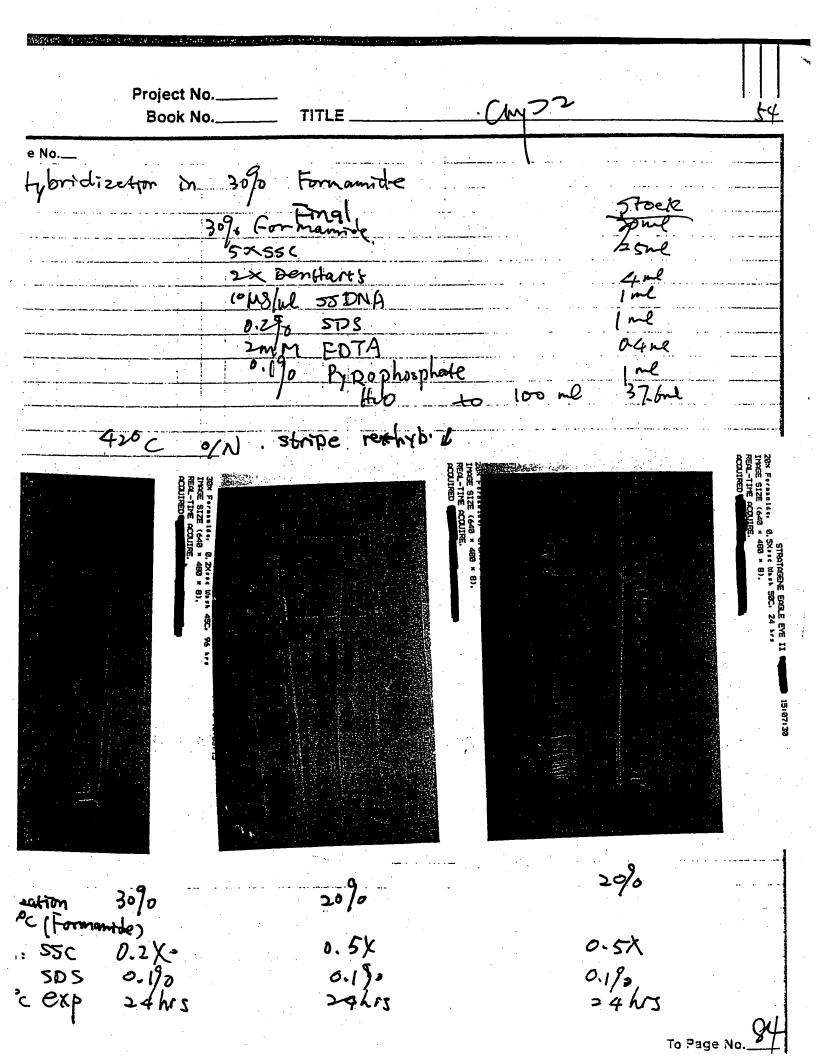
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| ysGluArgHisLeuLysGlnIleGlnIleGlyLeuAspGlnGlnAlaGluTyrLeuAs . 970 990 1010 XATCCTTCGACGAACACGAATGAAAATGAACACATGAAACAAATGAAACACAATCA XlnCysLeuGluGluAspGluAsnGluAspMetLysGluMetLysGluAsnGl 1030 1050 1070 ICGAAACCCTCAGAACCCACGCTCCCCCACCTCGAACTCAGCACTCAGCACTATTT fetLysProSerGluAlaArgValPrcGlnLeuSerSerLeuGluLeuArgArgTyrPh 1090 1110 1130 ACACCATACACAATTTCCTGAAACAAAACAAATACAGTGACTGCCTGC | ì | | | | <u>.</u> ! ממב | <u> </u> | | | | | | | | 1 |
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50 GTCGACCCACGCGTCCGGGTGTTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACC 110 AAGGCTGAGATGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTCA MetThrLeuLysTyrLeuTrpLeuValAlaLeuValAlaLeuTyrIleSer 130 150 170 CCCATCCAGICTCAGAACTGTGTGTATCTGGATCATACCATCTTGGAAAACATGAAACTT ProIleGlnSerGlnAsnCysValTyrLeuAspHisThrIleLeuGluAsnMetLysLeu 190 210 230 CTGAGCACCATCAGGACCACCTTTCCCTTAAGATGTCTAAAAGATATCACGGATTTTGAG LeuSerSerIleArgThrThrPheProLeuArgCysLeuLysAspIleThrAspPheGlu 270 290 TTTCCTCAAGAGATTCTGCTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACC PheProGlnGluIleLeuLeuTyrValGlnHisValLysLysAspIleLysAlaValThr TATCATATATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGGCG TyrHisIleSerSerLeuAlaLeuIleIlePheSerLeuLysAspSerIleSerLeuAla 390 ACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTGCAGCAAGCTCGA ThrGluGluArgLeuGluArgIleArgSerGlyLeuPheLysGlnValGlnGlnAlaArg 430 GAGTGCATGGTAGACGAGGAGAACAAGAACACGGAGGACGACAGTACATCACAACATCCT GluCysMetValAspGluGluAsnLysAsnThrGluGluAspSerThrSerGlnHisPro 490 530 CACTCAGAGGCTTCAAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGA HisSerGluGlyPheLysAlaValTyrLeuGluLeuAsnLysTyrPhePheArgIleArg 550 570 590 AAGITICCIGGIAAATAAGAAATACAGITTCIGIGCCIGGAAGATIGICGIGGIGGAAATA LysPheLeuValAsnLysLysTyrSerPheCysAlaTrpLysIleValValValGluIle 610 630 650 AGAAGATGITTCAGIATATTTTACAAACTACTCAACATGAATTGAGAATCATCCAGCTTC ArgArgCysPheSerIlePheTyrLysLeuLeuAsnMetAsnFnd 670 690 710 AAGCAAGAACTTAGATAGAAGTTGTGACTGCTCAAATGTCCCCAAGAACGCTTGATTCTA 730 750 AGGCTATTGCGAGTCTGCTGCTACACACTTCGGACGCAAGACTTTTCAAGGTCAGGGTTC 810 830 AAGGCAGTACAGTCAAAGGAAGTCTTATGTTAAGCAAAAGAAAAATTTCAGTGGAAAAGC 870 890 TAGCAGAAATGTCAACTTGTCAAAAAAACAACTTATGGATTATGGCATTGACGTTACTAG 910 930 950 CGC

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| Frame -2 Frame -3 One- more ATG @ 684 in frame with ATG starting from 602. To Pa | | | | | | |
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RESEARCH SUMMARY PAGE

mcDon 200 GN-000

Gene Name:

All Known Alias Gene Names:

| Iuman: Zhwxc00-00001-a1 at: Agp-22423-a1 | Member of the interferon family of proteins Name: Interferon-like protein. |
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| | |

Investigator(s):

Initial Date of Summary Preparation:

| Duanzhi Wen, Andrew Welcher, Michael Kelley | Initial invention disclosure filed This summary filled in on This summary filled in the filled in th |
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Description of Project:

This novel member of the interferon family of proteins is related to the beta, alpha, and omega subfamilies. As an interferon it would be expected to have anti-infective and anti-proliferative uses. Additionally, it might find use in the treatment of multiple sclerosis and other pathologies requiring immunomodulation.

Gene Nucleotide Sequence:

| ١ | | |
|----|------------|---|
| | Hum | an: |
| ĺ | | GCGTACGTA AGCTTAATTT AACAAAATTG GAAAAACCTA AACTATACTG |
| ١ | | TGCTCTGGTG ACCTAGCAAT CAAATAATCA CAGTCATTTG GTCAATGTCT |
| -[| 101 | ATGATTAACT CAATGAGACA GGATGTTTGG CTATAGCACC AGGTACAAAA |
| - | 151 | AATATATTT CATGAAGGAT CACTCCCTCT TATGTAATAG ATTTGGGTGA |
| - | 201 | GTGAGTGAGT GAGTGAGTGC ATGGACTCAC AGCTTTTGGC TTTCTGAAAT |
| 1 | 251 | ACCCTGCATC AGTCTTGTTA TGATGATTCC TTAGTGCTGG GATGGATCAT |
| 1 | 301 | CCAGGCATTT AAGGTAACAC GATGGTAATT CTTTGCTCAT TTTTCAGGGA |
| - | 351 | AAAAAAAAG TTATCACTTC CAAAGTCGGC ATAGTCACCC GAAGTAAAAA |
| 1 | 401 | AAAAAAAAAA AAAAAAAAGC CTCAGAGGCA AAGGAAAGGG GCCGCAACCT |
| 1 | 451 | TGGTTAACTG TGAAATGACG AATGAGAAAA CTCCTCCTGC TGAAGATATT |
| 1 | 501 | CAGGTATATA AAGGCACATG AAGGAAAACT CAAAACATCA TTGTCATATA |
| 1 | 551 | CACATCTTCT GGATTTTTTA GCTTGCAAAA AAAATGAGCA CCAAACCTGA |
| 1 | 601 | TATGATTCAA AAGTGTTTGT GGCTTGAGAT CCTTATGGGT ATATTCATTG |
| 1 | 651 | CTGGCACCCT ATCCCTGGAC TGTAACTTAC TGAACGTTCA CCTGAGAAGA |
| 1 | 701 | GTCACCTGGC AAAATCTGAG ACATCTGAGT AGTATGAGCA ATTCATTTCC |
| ١ | 751 | TGTAGAATGT CTACGAGAAA ACATAGCTTT TGAGTTGCCC CAAGAGTTTC |
| 1 | 801 | TGCAATACAC CCAACCTATG AAGAGGGACA TCAAGAAGGC CTTCTATGAA |
| 1 | 851 | ATGTCCCTAC AGGCCTTCAA CATCTTCAGC CAACACACCT TCAAATATTG |
| ١ | 901 951 | GAAAGAGAGA CACCTCAAAC AAATCCAAAT AGGACTTGAT CAGCAAGCAG AGTACCTGAA CCAATGCTTG GAGGAAGACG AGAATGAAAA TGAAGACATG |
| 1 | 1001 | |
| ١ | 1051 | |
| 1 | 1101 | |
| ł | 1151 | |
| 1 | 1201 | |
| ١ | 1251 | TTTCTCCTTC TCCTCCTCCA TCTTCTTTTT AAGGATTGTT GTGCTGTCCT |
| 1 | 1301 | GTAAGCCTGT CCTCAGTTGG ACTGGTAGCC TCGGAACATC AGGGACACTC |
| ٠. | | Annual deliging instance 1600Myours (000minute) |

ACCTCTCTAA GGAGAGGTAA TGCCAACCAT CCTCAGGGTG ACCAAGAGTC TCCTTAGAAA GTCTTTAAGA CATTTTTAAA GGAATAAGAT TCCCTCTCCG 1401 TCTTCTTCTA TTCTCTTTG CTCTTTTCTG TGGCCATTTT GAAAGAGCTT 1451 1501 TGCTATATAT ACCACCTGTG GACTTCACCA AGACAATGGC TAGAGGATAG 1551 GGAGCAGAGA ATGTTGCAAA ATGGTAACAT TTCAATGACT TAACTGTTTT GCTGCCAAGG TTGCTTATCC TATGAAAATT CAGCACATTA AAAGAGCTTA 1601 TACATGCTCC CTAGAGTCAA TACTCTTGCA TTTTCCCCCT CCTGCTCGGG 1651 1701 GGGAAAAAGG TTGACATTTC TGGCCCATTT CCTTCTCAGC TTGGTTTGTT TGAATTGATG CTTGTGGAAT GGTATTTCAT TACTTTAAGA GTGAAGATCC ATAGTGAAAT TGGATGGATG GTTGAATTAG ACGACCATTA AGCTTGGATC 1751 1801 CTCTAGAGCG GCCGCCGACT AGTGAGCTCG TCGACCCGGG AATT 1851 Rat: 1 GGGTGTTGTA GATATTTTC CTTTGGAAGA AATACTGAGC ACCAAGGCTG 51 AGATGACACT GAAGTATTTA TGGCTGGTGG CCCTCGTGGC TCTATACATT 101 TCACCCATCC AGTCTCAGAA CTGTGTGTAT CTGGATCATA CCATCTTGGA 151 AAACATGAAA CTTCTGAGCA GCATCAGGAC CACCTTTCCC TTAAGATGTC 201 TAAAAGATAT CACGGATTTT GAGTTTCCTC AAGAGATTCT GCTGTACGTC 251 CAGCATGTGA AAAAGGACAT AAAGGCAGTC ACCTATCATA TATCTTCTCT 301 GGCGCTAATT ATTTTCAGTC TTAAAGACTC CATCTCCCTG GCGACAGAGG 351 AACGCTTGGA ACGTATCAGA TCGGGACTTT TCAAACAAGT GCAGCAAGCT 401 CGAGAGTGCA TGGTAGACGA GGAGAACAAG AACACGGAGG AGGACAGTAC 451 ATCACAACAT CCTCACTCAG AGGGCTTCAA GGCAGTCTAC CTGGAATTGA 501 ACAAGTATTT CTTCAGAATC AGAAAGTTCC TGGTAAATAA GAAATACAGT 551 TTCTGTGCCT GGAAGATTGT CGTGGTGGAA ATAAGAAGAT GTTTCAGTAT 601 ATTTTACAAA CTACTCAACA TGAATTGAGA ATCATCCAGC TTCAAGCAAG 651 AACTTAGATA GAAGTTGTGA CTGCTCAAAT GTCCCCAAGA ACGCTTGATT 701 CTAAGGCTAT TGCGAGTCTG CTGCTACACA CTTCGGACGC AAGACTTTTC 751 AAGGTCAGGG TTCAAGGTAG TACAGTCAAA GGAAGTCTTA TGTTAAGCAA 801 AAGAAAAATT TCAGTGGAAA AGCTAGCAGA AATGTCAACT TGTCAAAAAA 851 ACAACTTATG GATTATGGCA TTGACGTTAC TAGCAAAAA AATAAAACAA 901 АААААААСАА ААА

Gene Amino Acid Sequence:

Human:

- 1 MSTKPDMIQK CLWLEILMGI FIAGTLSLDC NLLNVHLRRV TWQNLRHLSS MSNSFPVECL RENIAFELPQ EFLQYTQPMK RDIKKAFYEM SLQAFNIFSQ
- HTFKYWKERH LKQIQIGLDQ QAEYLNQCLE EDENENEDMK EMKENEMKPS 101
- EARVPOLSSL ELRRYFHRID NFLKEKKYSD CAWEIVRVEI RRCLYYFYKF 151 201 TALFRRK*

Rat:

- MTLKYLWLVA LVALYISPIQ SQNCVYLDHT ILENMKLLSS IRTTFPLRCL
- KDITDFEFPQ EILLYVQHVK KDIKAVTYHI SSLALIIFSL KDSISLATEE 101 RLERIRSGLF KOVOQARECM VDEENKNTEE DSTSQHPHSE GFKAVYLELN
- KYFFRIRKFL VNKKYSFCAW KIVVVEIRRC FSIFYKLLNM N*

Figure Containing cDNA and Amino Acid Sequences:

Human:

Sequence Analysis of Human IFN-novel

```
CGCGTAGGTAAGCTTAATTTAACAAATTGGAAAAACCTAAACTATACTGTGGTG
     ACCTAGGAATCAAATAATCACACTCATTTGGTCAATCTCTATCATTAACTCAATGAGACA
                                                         120
 121
     CONTETTTGGCTATAGCACCACCTACAAAAATATATTTTCATCAACGATGACTCCCTCT
                                                         180
 181
     TATETAATAGATTTEGGTCACTCACTCACTCACTGAGTGCATGCACTCACACCTTTTGGC
                                                         210
                                                         300
 301
                                                         160
     420
 421
     CTCACACCCAAAGGAAAGGGGCCCCAACCTTCGTTAACTGTGAAATGACGAATCACAAAA
     CTCCTCCTCCAGATATTCACCTATATAAACCCCAGATGAAGCAAAACTCAAACATCA
                                                         540
     TTGTCATATACACATGTTCTGGATTTTTTAGCTTCCAAAAAAATGAGCACCAAACCTGA
                                                         600
     660
     ATCCCTGCACTOTAACTTACTGAACGTTCACCTCACAAAGAGTCACCTGGCAAAATCTCAG
          <u>ochtluvalarvtwonle</u>
     ACATCTGAGTAGTATGAGCAATTCATTCCTGTAGAATGTGTACGAGAAACATACCTTT
                                                         780
          S-S M S N S P S V R C L R E M I A P
     TGAGTTCCCCCAAGAGTTTCTCCAATACACCCAAGGTATGAAGAGGGACATCAAGAAGCC
 701
                                                         840
     ELPQEPLOYTOPK KRDIKKA
 61
     CTTCTATCAAATCTCCCTACAGCCCTTCAACATCTTCAGCCAACACCCTTCAAATATTC
 841
        Y E M B L Q A Y M I P B Q H T F K Y W
     ARLEGIQICLD Q Q A E Y L N
     CERATOCTTCCACCALGACGAGAATGAAAATGAACACATCAAAGAAAATGAAAGACAATCA
     CATGAAACCCTCACAACCCACCCTCCCCCCCAGCTGAGCAGCTCCCAACTCAGGAGATATTT
     M K P S B A R V P Q L S S L E L R R Y P
                                                        160
1081
     CCACAGGATAGACAATTTCCTKAAAGAAAAGAAATACAGTCACTCTCCCTTGGGAGATTGT
1141
     ceraticeaaatcacaacatcittutattacittiacaattiacaccicitaticaggag
181
1201
                                                        1260
201
1261
1321
     ACTGGTACCGTCGGRACATCAGGGACACTCACCTCTGTAAGGAGAGGTAATCCCAACCAT
1381
     CCTCAGCGTGACGAAGAGTGTCCTTAGAAAGTTTTAAAGAATTTTTAAAGGAATAAGAT
                                                       1440
1441
1501
                                                       1560
1561
    ATGTTCCAXAATCCTAACATTTCAATGACTTAACTCTTTTCCTGCCAAGGTTGCTTATCC
                                                       1620
1621
    TATGRAMATTCACCACATTAAAAGAGCTTATACATGCTCCCTAGACTCAATACTCTTGCA
                                                       1680
1681
     TITTCCCCCCCCCCCCCCCCCCCCCAAAAGGTTGACATTCCTCCCCCCCATTCCTTCTCAGC
                                                       1740
1741
    TTGGTTTGTTTGAATTGATCCTTCTCCAATGGTATTTCATTACTTTAACACTGAAGATCC
                                                       1800
1801
    ATAGTUAATTGGATGGATGGTTCAATTAGAGGACCATTAACCTTCGATCCTCTAGAGGG
                                                       1860
1861 GCCGCCGACTACTGACCTCCTCCACCCCGGGAATT
                                                        1894
```

A human gene which encodes a novel protein of 207 amino acids was isolated by screening the human genomic DNA library using a rat cDNA clone. The deduced amino acid sequence of this novel gene is indicated below the first nucleotide of each codon, and the termination codon is marked with an asterisk. The protein starts with cysteine, and the signal peptide is underlined. This novel protein is 27% identical to human IFN-β.

Rat:

| 1 | | | | | | | | | | | | | | | | | | M | <u>T</u> | L |
|------------|-----|-------------|-----|------|----------|------------|------|-------|----------|-------|-------|------|------|-----------|--------------|------|------|-------|----------|------------|
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| 61 | | | | | | | | | | | | | | | | | | | | gaa |
| 4 | K | Υ_ | L | W | <u>L</u> | ٧. | Α | 1 | <u>v</u> | A | Ŀ | Y | I. | 3 | ₽. | I | ؎ | 3 | 0 | N |
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| L21 | | | | | | | | | | | | | | | | | | | | GAC |
| 24 | C | V | Y | L | D | H | Ŧ | I | Ľ, | E | N | M | K | L. | L | 8 | S | T | R | T. |
| 181 | CAC | لملن | TCC | ਵਾਦਾ | 'AAG | ATG | TCT | AAA | AGA | TAT | CAC | GGA | TII | TGA | GTT | TCC | 1CA | AGA | GAT | TCT |
| 44 | - π | 7 | P | L | R | C | L | K | D | ·I | T | D | P | E | ŕ | ₽ | Q | E | I | L |
| | • | • | • | | | - | - | | _ | ٠. | | | | | | | • | | | |
| 241 | GCI | GTA | CGI | CCA | GCA | TGI | GAA | AAA | GGA | CAI | AAA | GGC | AGT | CAC | CTA | TCA | TAT | ATC | TIC | TCT |
| 64 | | | | Q | | | | | | | | A | | | | | | ŝ | | L |
| | | | | | | | | | | | | | | | | | | | | • |
| 301 | GGC | GCI | AAT | TAT | TTI | CAC | TCI | TAN | AGA | CIC | CAI | CTC | CCT | GGC | GAC | AGA | GGA | ACG | CTI | GGA |
| 84 | A | L | I | I | ·F | 5 | Ļ | K | D | S | I | 9 | L | A | T | E | E | R | L | E |
| | | | | | | | | | | | | | | | | | • | | | • |
| 361 | | | | | | | | | | | | | | | | | | | | ÇGA |
| 104 | R | I | R | S | Ğ | L | P | K | Q | V | Q | Q | A | R | E | C | M | V | D | E |
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| 121 | | | | | | | | | | | | | | | | | | | F | ÇAA |
| 124 | E | N | K | N | T | E | E | D, | 3 | T | 5 | · | л | | п | 3 | | J | • | <u>.</u> |
| 401 | ~~~ | · > <= | - | ~~ | V2G2 | . अग्रच | ~^A | (7) 2 | CITY | ALAL. | | Y'ac | . בב | • Y'AC | LAA! | GTI | CCI | GČI | AAJ | TAA |
| 181 144 | A | | | | | | | | | | | | | | | | | V | | K |
| 144 | A | ٧ | • | | _ | - | | - | • | • | • | | _ | | - | | | | | |
| 541 | CAZ | የ ተፈ | CAC | 7111 | CTC | TGC | CTC | GAZ | GAT | TG/ | NGG/I | KG T | (CC) | AAT | ΑΛ | AAC | ATC | TI | ĊX | TAT |
| 164 | K | Y | s | F | C | A | W | K | I | V | V | V | E | I | R | R | C | P | | I |
| | • | - | _ | | | | | | | | , | | | | | | . • | | | • |
| 501 | ATT | 112 | دی | VACT | 'AC' | CA | CAI | œλ. | VIIX | :AG | XX. | XX. | CAC | CT | CN | GCI | LAGI | VACT | TAC | ata |
| 184 | | | | L | | | | | | | | | | | | | | | | |
| | _ | | | | | | | | | | , | | | • | | | • | | | |
| 661 | GAZ | GI | GIV | ACT | IGC. | CA | VAIX | TCC | CC2 | VAC | ACC | CT | (CA) | TC | ' እእር | GC1 | 'AT | GCC | ΑĊ | CIG |
| 721 | CIC | CTA | CAC | AC | TCC | GAC | :GCJ | VACI | ĊT | TT | 'AAC | GIX | .AG | GI | CN | VGGC | 'AG' | CAC | CIX | AAA |
| | | | | | | | | | | | | | | | | ~~ | 433 | N TOP | 7 | VACT |

Cloning Information:

The rat sequence was cloned from a rat placenta cDNA library as part of an EST project and was identified by computer analysis as being a novel member of the interferon family of proteins. Briefly, rat embryo day 17 [E17] placenta mRNA was isolated by standard methods (unnecessary information) (Chomczynski, P. and Sacchi, N., Anal. Biochem. 162, 156, 1987). cDNA was synthesized using the SuperScript Plasmid cDNA kit supplied by GIBCO/BRL and subcloned into the pSPORT1 (GIBCO/BRL) vector into the Sal 1 and Not 1 restriction sites.

Cloning of Human IFN-like gene:

Multiple attempts to clone the human IFN-like gene from a variety of human tissue cDNA libraries failed to yield positive clones. However, a human tissue Northern Blot

hybridized with a PCR-generated radioactive rat probe revealed an 1.8 kb Hind III fragment in certain batches of human pancreas mRNA. Attempts to clone this corresponding message in a pancreas cDNA library failed to recover any positive clones.

Examination of the genomic structures of known IFNs revealed that IFN, especially the members in the IFNa family, all share a unique intronless genomic structure. Therefore, screening of human genomic DNA might yield the complete human IFN-like gene. We started with 1×10^6 human lambda genomic clones (Stratagene, Cat. No. 946206) for primary screening at a density of 50, 000 clonies / plate (unnecessary information). Nitrocellulose filters (unnecessary information) (S&S) were prepared by standard techniques (Molecular Cloning, A Laboratory Manual, Sambrook, Fritsch, and Maniatis editors).

The following conditions were used.

- Prehybridization and hybridization conditions: 30% formamide, 5x SSC, 2x Denhart's, 10μg/ml Salmon sperm DNA, 0.2% SDS, 2mM EDTA and 0.1% pyrophosphate.
 Hybridization was conducted overnight at 42°C. The washings were done under following conditions: 1x SSC, 0.1%SDS at room temperature for 30-60 minutes followed by 0.2x SSC and 0.1%SDS at 55°C for 15 minutes.
- Generation of radioactive PCR probe (unnecessary information): rat cDNA full-length fragment 20ng, primer 1795-01 and 1795-02, 20 pmol each, 1mmol dNTP (dCTP @ 0.01mmol), 32P-dCTP 5 ml and 4mM MgCl2. Reaction condition: denature at 94 °C for 30sec, anneal at 60 °C for 30sec and elongate at 72 °C for 1 minute. The reaction is repeated for a total of 45 times. Simultaneously a "cold" PCR reaction is performed under exact condition except the dNTP mix is dCTP balanced. The radioactive probe was purified by Quick Spin G-50 column and boiled at 100 °C for 10 minutes before chilling on dry ice for 20 minutes. The probe is usually 5x10⁵ cpm/μl.

Three positive clones were recovered after primary, secondary screening and subsequently purified to homogeneity. The lambda phage DNA was prepared by a solid plate culture method. The NotI insert from these clones were excised out and ligated into pSport (GIBCO BRL) vector and transformed into DH10 E. coli strain. The transformants were prepared by Qiagen Spin Column plasmid prep kit. The plasmid DNA was then digested with HindIII. The digested fragments were resolved on agarose gel and transferred to a nylon membrane for Southern Blot analysis. The analysis was conducted under the same condition genomic screening was carried out. The corresponding fragment recognized by "hot" rat probe was then subloned in pSport vector for sequencing analysis. According to the HindIII digestion pattern, we determined these three independent clones were likely to contain identical genomic insert. The sequencing analysis confirmed our speculation. This 1.8kb HindIII fragment contains an open reading frame of 624 base pairs that has 64% similarity to the sequence of rat mrpe3-00078-F6-Wz. In terms of similarity in amino acid sequence, the human sequence is 40.5% identical to and 50% similar to that of rat. All 5 predicted Cysteine residues were perfectly aligned with those in rat protein sequence. Moreover, the human sequence is predicted to contain a signal peptide and cleavage site. The human IFN-like protein is strongly predicted to resemble a secreted cytokine molecule (91% probability).

Homology of Multiple Gene Family Members:

Amino Acid Sequence Alignment of Human IFN-novel, Rat IFN-novel and Human IFN-β

| • | | |
|---|--|--------------------------|
| Human IFN-novel Human IFN-bets Rat IFN-novel | TON THE LIMIT COT SLATING STATE OF THE SHARLEST STATE OF THE SHARL | 36 43 32 50 |
| Consensus | L 8, 4 £ £ | |
| Ruman IPN-novel Human IFN-beta Rat IFN-novel Consensus | CALTINESMS METPVECLER MILES FOR LOTTING CLATTERS CALTINES CALTURED | 86 85 83 |
| Numan IFN hovel Ruman IFN bets Rat IFN novel Consensus | OAPNI T PKY-WKE | 121 122 118 150 |
| Ruman IFN-novel Rat IFN-novel Consensus | CLESORSINE DOCLOCENIM KP ARVPO LESIKLIAYF HRIDWYLD LESKI ZO FROKI MESIRIL YY GRILAYIM CMVDEEN | 170 157 163 200 |
| Numan IFH-novel Ruman IFH-beta Rat IFH-novel Consensus | TALPER K LTOYLENG - LTOYLENG - LLENGI | 201 187 191 231 |

Human IFN-novel is most close to human IFN-β, with 30% identity. Four out of five cysteine residues are conserved between them.

Presence and Distribution of mRNA in Different Tissues:

Northern blot analysis detected IFN-like mRNA in several different stages of mouse and rat embryos. Northern blots used RNA isolated as above. The full-length rat cDNA was used as a probe. Prehyb conditions were 40 % formamide, 5X SSC, 1 mM EDTA, 0.1 % SDS, for 4 h at 42°C. Hyb conditions were the same as above except were done overnight at 42°C. Blots were washed with 0.2x SSC, 1 mM EDTA, and 0.1% SDS for 30 min at 60°C.

RT-PCR (conditions are not necessary - standard technology) identified IFN-like mRNA in the following human tissues: pancreas, small intestine, prostate, uterus, thyroid, and placenta.

Recombinant Protein Expression:

Production of human and rat IFN-like protein in E. coli:

Waiting on data from Karen Sitney. However, the E. coli protein did not appear to be folded correctly and has not yet generated any biologically active material.

Production of human and rat IFN-like protein in a mammalian expression system:

Several versions of the human and rat IFN-like protein have been produced in a mammalian expression system (either CHO or 293 cells). The proteins synthesized were either the native protein itself, or a native protein-Fc fusion. Some of the Fc fusion constructs contained a cleavage site which allows the native protein to be released from the Fc portion after being produced in the conditioned media of CHO cells.

PCR amplification of IFN-like molecule:

PCR primers were selected to amplify the coding sequence of rat/human IFN-like molecule:

Rat IFN-Like Molecule primers:

IFN-Like molecule Fc-fusion:

1847-77 CCC <u>AAG CTT</u> ACC ATG ACA CTG AAG TAT TTA TG

Forward primer: Hind III site plus ATG

1847-78 AAG GAA AAA A<u>GC GGC CGC</u> ATT CAT GTT GAG TAG

Reverse primer: Not I site and no stop codon for Fc fusion

Soluble IFN-like molecule:

1896-56 ACG CGT CGA CTC ATC AAT TCA TGT TGA GTA GTT TG

Reverse primer: Sal I site plus 2 stop codons (for pDSRa cloning).

1896-57 AAG GAA AAA A<u>GC GGC CGC</u> TCA TCA ATT CAT GTT GAG TAG

Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

Human IFN-like primers:

Soluble human IFN-like primers:

1954-45 ACG CGT CGA CTT ATT ATT TCC TCC TGA ATA G

Reverse prime: Sal I site plus 2 stop codons (for pDSRa cloning).

1954-46 AAG GAA AAA AGC GGC CGC TTA TTA TTT CCT CCT GAA TAG AGC

Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

Human IFN like-Fc fusion primers:

1955-44 CCC AAG CTT ACC ATG AGC ACC AAA CCT GAT ATG

Forward primer: Hind III site with 1st ATG

1954-47 CCC AAG CTT ACC ATG ATT CAA AAG TGT TTG TGG C

Forward primer: Hind III site with 2nd ATG

1954-48 AAG GAA AAA AGC GGC CGC GCG GCC CTC GAT TTT CCT CCT GAA TAG

AGC TGT AA

Reverse primer: Not I site, no stop codon with Factor Xa cleavage site and Fc fusion 1954-49 AAG GAA AAA A<u>GC GGC CGC</u> TTT CCT CCT GAA TAG AGC TGT AA

Reverse primer: Not I site and no stop codon for Fc fusion

PCR Reaction:

Rat:

Reaction Mixture: template 20 ng, 1847-77 and 1847-88 or 1896-56/57, 20 pmol each, 1mmol dNTPs, 4mM MgCl2, 1X PCR buffer, 5u Taq polymerase.

Reaction condition: 2 cycle-linked PCR.

94 °C for 30 °C sec, 50 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94

°C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

Human interseron-like protein PCR conditions:

Reaction Mixture: template 20 ng, 1955-44 and 1954-45or 1954-46 (soluble form) or 1945-48/49 (Fc fusion), 20 pmol each, 1mmol dNTPs, 4mM MgCl2, 1X PCR buffer, 5u Taq plymerase.

Reaction condition: 2 cycle-linked PCR.

94 °C for 30 °C sec, 48 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94 °C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

While 1955-44 primer generates an ORF using first Met in the coding region, a separate PCR with 1954-47 to obtain an insert using 2nd downstream Met was also generated. But in terms of secretion efficiency, when tested in 293 EBNA transient transfection, there was no detectable difference could be defined.

For both rat and human, the PCR products were purified by Qiagen PCR purification spin column and subjected to restriction digestion by respective enzymes (HindIII and NotI (pCEP4) or SalI(pDSRα)). After digestion, the fragment was purified from agarose gel with Qiagen gel purification spin column. The purified fragment was quantified and ligated into pCEP4 (for native form), pCEP4-Fc (for Fc form) or pDSRα (native form or Fc form) vectors respectively. The ligation was transformed into DH10. The transformants were picked for miniprep and subsequent sequencing verification. Accuracy of each cloning fragment was verified by sequencing including the Fc junction sequence. The clone was then maxi-prepared for tissue culture transfection experiments. The IFN-Fc fragment in pCEP4-Fc vector can be released by cutting this vector with HindIII and SalI and re-ligated this fragment into pre-digested pDSRα to yield a vector suitable to transfect CHOD cells.

Transfection:

- Protocol for transfeciton into 293 EBNA and CHO cells with lipofectin was adopted from the one used by Jin Cao. Same protocol was used to generate both transient and stable transfectants.
- A commercial available calcium phosphate transfection kit was used in CHO cell stable transfection (protocol is attached).
- A CHO cell transfection and selection protocol from Yi Luo was utilized, except calcium
 phosphate transfection procedure, which has a commercially available kit.

In general, lipofectin transfection yields more stable transfection colonies. Those colonies express comparable level of secreted proteins as those picked from calcium phosphate method.

Generate conditioned media containing recombinant protein.

In order to conduct functional studies on this interferon-like molecule, large quantity of conditioned media (CM) were generated from a pool of hygromycin selected 293 EBNA clones. The cells were cultured in Nunc Triple Flask (500cm) to 80% confluence before switching to serum free media for a week before harvesting. The CM was then sent to purification with protein A affinity chromatography. The purified protein was then used to generate a rabbit polyclonal antibody and to test for in vitro activities. The processing of signal peptide as well as partial amino acid sequence was verified by peptide sequencing.

Purification of human IFN-like-Fc

Conditioned media from CHO cells expressing hulfLM-Fc was thawed and 0.2µm filtered. The filtered material was loaded onto a Protein G column that was previously equilibrated with PBS, pH 7.0. After loading, the column was washed with PBS until the absorbence at A₂₈₀ reached baseline. The protein was eluted from the column with 0.1M Glycine-HCl pH 2.7 an dimmediately neutralized with 1M Tris-HCl pH 8.5. Fractions containing hulf-LM-Fc were pooled and dialyzed into PBS and stored at -70°C.

Factor Xa cleavage of human IFN-like-Fc

The human IFN-like-Fc construct has a Factor Xa cleavage site (IEGR) inserted between the Fc and huIFLM. This site is cleaved with restriction protease factor Xa. The human IFN-like-Fc in PBS was dialyzed into 50mM Tris-HCl, 100mM NaCl, 2mM CaCl, pH 8.0. The Factor Xa was added to the dialyzed protein at 1/100 (w/w). The sample was incubated overnight at room temperature.

Abs (available, ordered, proposed):

| 1. Polyclonal: | |
|--|---|
| Polyclonal antii CHO cells (from the proteins as o | bodies were prepared using both rat and human proteins produced in E. coli arm above) using standard immunological techniques. Antisera were positive for determined by Western blot analysis (standard techniques) |
| 2. Monoclonal: | |
| None. | |
| 3. Peptides: | |
| None. | |

Phenotype and/or Biological Activity:

| 1. Transgenic / (pending / analyzed) | |
|--------------------------------------|--|
|--------------------------------------|--|

| Because the lack of a phe from this experiment. Fur proteins' biological activi | enotype constitutes a 'negative' result no conclusions can be drawn or ther testing will be required to determine any or all of IFN-like ities in vivo. |
|---|---|
| 2. in vivo assays: | (available, used, proposed) |
| Not done. | |
| 3. in vitro assays: | (available, used, proposed) |

References:

Nothing specifically published on this gene. Lots of references for the interferon family.

Genomic DNA Sequence (i.e. including all introns and exons):

The human gene was cloned from genomic DNA. The attached sequence (above) comes from genomic DNA and includes the coding region which is found in one exon, and the flanking regions.

Ortholog DNA Sequences:

Human and rat sequences cloned.